

CLAIMS

We claim:

1. A purified and isolated nucleic acid sequence encoding a levopimaradiene synthase.
2. A purified and isolated nucleic acid sequence comprising SEQ.ID.NO:1, SEQ.ID.NO:32, SEQ.ID.NO:34, SEQ.ID.NO:36 or SEQ.ID.NO:38.
3. A purified and isolated nucleic acid sequence comprising SEQ.ID.NO:34.
4. A purified and isolated nucleic acid sequence comprising SEQ.ID.NO:36.
5. An expression vector comprising an isolated and purified nucleic acid sequence encoding a levopimaradiene synthase under the control of a promoter operable in a host cell.
6. The expression vector of claim 5, wherein said promoter is an inducible promoter.
7. The expression vector of claim 6, wherein said inducible promoter is GAL1.
8. The expression vector of claim 5, wherein said host cell is a prokaryote.
9. The expression vector of claim 8, wherein said prokaryote is *Escherichia coli*.
10. The expression vector of claim 5, wherein said host cell is a eukaryote.
11. The expression vector of claim 10, wherein said eukaryote is a yeast.
12. The expression vector of claim 5, wherein said nucleic acid sequence comprises SEQ.ID.NO:1, SEQ.ID.NO:32, SEQ.ID.NO:34, SEQ.ID.NO:36 or SEQ.ID.NO:38.
13. The expression vector of claim 12, wherein said promoter is an inducible promoter.
14. The expression vector of claim 13, wherein said inducible promoter is GAL1.
15. The expression vector of claim 12, wherein said host cell is a prokaryote.
16. The expression vector of claim 15, wherein said prokaryote is *Escherichia coli*.

17. The expression vector of claim 12, wherein said host cell is a eukaryote.

18. The expression vector of claim 17, wherein said eukaryote is a yeast.

19. As a composition of matter, an isolated polypeptide comprising an amino acid sequence of a levopimaradiene synthase.

20. As a composition of matter, an isolated polypeptide comprising an amino acid sequence of SEQ.ID.NO:2, SEQ.ID.NO:33, SEQ.ID.NO:35, SEQ.ID.NO:37 or SEQ.ID.NO:39.

21. As a composition of matter, an isolated polypeptide comprising an amino acid sequence of SEQ.ID.NO:35.

22. As a composition of matter, an isolated polypeptide comprising an amino acid sequence of SEQ.ID.NO:37.

23. An expression vector comprising an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence of a levopimaradiene synthase.

24. An expression vector comprising an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ.ID.NO:2, SEQ.ID.NO:33, SEQ.ID.NO:35, SEQ.ID.NO:37 or SEQ.ID.NO:39.

25. A unicellular organism comprising a purified and isolated nucleic acid sequence encoding a levopimaradiene synthase.

26. The unicellular organism of claim 25, wherein said nucleic acid sequence encoding said levopimaradiene synthase comprises SEQ.ID.NO:1, SEQ.ID.NO:32, SEQ.ID.NO:34, SEQ.ID.NO:36 or SEQ.ID.NO:38.

27. The unicellular organism of claim 25, wherein said nucleic acid sequence further comprises an expression vector.

28. The unicellular organism of claim 27, wherein said expression vector comprises an inducible promoter.

29. The unicellular organism of claim 28, wherein said inducible promoter is GAL1.

30. The unicellular organism of claim 25, wherein said nucleic acid sequence encoded said levopimaradiene synthase containing a deletion in the N-terminal sequence.
31. The unicellular organism of claim 25, wherein said organism is *Saccharomyces*, *Escherichia coli*, *Candida albicans* or *Kluyveromyces lactis*.
32. The unicellular organism of claim 25, wherein said organism is *Escherichia coli*.
33. The unicellular organism of claim 25, wherein said organism is *Saccharomyces cerevisiae*.
34. A yeast host cell comprising a vector, wherein said vector comprises a purified and isolated nucleic acid sequence of claim 2 under control of a promoter operable in said yeast host cell.
35. A yeast host cell comprising a vector, wherein said vector comprises an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence of claim 20 under control of a promoter operable in said yeast host cell.
36. A plant host cell comprising an isolated and purified nucleic acid sequence of claim 2 under control of a promoter operable in said plant host cell.
37. The plant host cell of claim 36, wherein said plant is *Ginkgo biloba*.
38. A unicellular organism comprising an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence of a levopimaradiene synthase.
39. The unicellular organism of claim 38, wherein said amino acid sequence comprises SEQ.ID.NO:2, SEQ.ID.NO:33, SEQ.ID.NO:35, SEQ.ID.NO:37 or SEQ.ID.NO:39.
40. The unicellular organism of claim 38, wherein said polypeptide contains a deletion in an N-terminal sequence.
41. The unicellular organism of claim 38, wherein said organism is *Saccharomyces*, *Escherichia coli*, *Candida albicans* or *Kluyveromyces lactis*.
42. The unicellular organism of claim 38, wherein said organism is *Escherichia coli*.

43. The unicellular organism of claim 38, wherein said organism is *Saccharomyces cerevisiae*.

44. A method of producing a ginkgolide in a cell, comprising the steps of:

obtaining a cell of the unicellular organism of claim 25;
culturing said cell under conditions wherein said cell produces said ginkgolide; and
removing said ginkgolide from said culture of cells.

45. The method of claim 44, wherein said nucleic acid sequence encoding said levopimaradiene synthase comprises SEQ.ID.NO:1, SEQ.ID.NO:32, SEQ.ID.NO:34, SEQ.ID.NO:36 or SEQ.ID.NO:38.

46. A method of producing levopimaradiene in a cell, comprising the steps of:

obtaining a cell of the unicellular organism of claim 25;
culturing said cell under conditions wherein said cell produces levopimaradiene;
and
removing said levopimaradiene from said culture of cells.

47. The method of claim 46, wherein said nucleic acid sequence encoding said levopimaradiene synthase comprises SEQ.ID.NO: 1, SEQ.ID.NO: 32, SEQ.ID.NO: 34, SEQ.ID.NO: 36 or SEQ.ID.NO: 38.

48. A method of producing a ginkgolide in a cell, comprising the steps of:

obtaining a yeast cell of claim 34;
culturing said cell under conditions wherein said cell produces said ginkgolide; and
removing said ginkgolide from said culture of cells.

49. A method of producing levopimaradiene in a cell, comprising the steps of:

obtaining a yeast cell of claim 34;
culturing said cell under conditions wherein said cell produces levopimaradiene;
and

removing said levopimaradiene from said culture of cells.

50. A method of producing levopimaradiene in a cell, comprising the steps of:

obtaining a cell of claim 38;

culturing said cell under conditions wherein said cell produces levopimaradiene;

and

removing said levopimaradiene from said culture of cells.

51. The method of claim 50, wherein said amino acid sequence encoding said levopimaradiene synthase comprises SEQ.ID.NO:2, SEQ.ID.NO:33, SEQ.ID.NO:35, SEQ.ID.NO:37 or SEQ.ID.NO:39.

52. A method of producing a ginkgolide in a cell, comprising the steps of:

obtaining a cell of the unicellular organism of claim 40;

culturing said cell under conditions wherein said cell produces said ginkgolide; and
removing said ginkgolide from said culture of cells.

53. A method of producing levopimaradiene in a cell, comprising the steps of:

obtaining a yeast cell of claim 35, wherein said cell further comprises an increase in the effective amount of geranylgeranyl diphosphate;

growing a culture of said cells under conditions wherein said cell produces said geranylgeranyl diphosphate and said levopimaradiene; and

removing said levopimaradiene from said culture of cells.

54. A ginkgolide, wherein said ginkgolide is obtained from production in a unicellular organism, comprising a purified and isolated nucleic acid sequence encoding levopimaradiene synthase.

55. A ginkgolide, wherein said ginkgolide is obtained from production in a unicellular organism, comprising a purified and isolated nucleic acid sequence of SEQ.ID.NO:1, SEQ.ID.NO:32, SEQ.ID.NO:34, SEQ.ID.NO:36 or SEQ.ID.NO:38.

56. A ginkgolide, wherein said ginkgolide is obtained from production in a unicellular organism, wherein said organism comprises an expression vector comprising an isolated and purified nucleic acid sequence encoding a levopimaradiene synthase under the control of a promoter operable in said organism.

57. A ginkgolide, wherein said ginkgolide is obtained from production in a unicellular organism, wherein said organism comprises an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ.ID.NO:2, SEQ.ID.NO:33, SEQ.ID.NO:35, SEQ.ID.NO:37 or SEQ.ID.NO:39.

58. A ginkgolide, wherein said ginkgolide is obtained from the method of claim 44.

59. A ginkgolide, wherein said ginkgolide is obtained from the method of claim 48.

60. A ginkgolide, wherein said ginkgolide is obtained from the method of claim 52.

61. As a composition of matter, a purified and isolated nucleic acid sequence comprising SEQ.ID.NO:5, SEQ.ID.NO:6, SEQ.ID.NO:7, SEQ.ID.NO:8, SEQ.ID.NO:9, SEQ.ID.NO:10, SEQ.ID.NO:11, SEQ.ID.NO:12, SEQ.ID.NO:29, SEQ.ID.NO:30, SEQ.ID.NO:31 or SEQ.ID.NO:40.

62. A transgenic plant, wherein said plant comprises an isolated and purified nucleic acid sequence encoding a levopimaradiene synthase under control of a promoter operable in said transgenic plant.

63. The plant of claim 63, wherein said nucleic acid sequence encoding said levopimaradiene synthase comprises SEQ.ID.NO: 1, SEQ.ID.NO: 32, SEQ.ID.NO: 34, SEQ.ID.NO: 36 or SEQ.ID.NO: 38.

64. The plant of claim 63, wherein said plant is *Ginkgo biloba*.

65. A seed of the transgenic plant of claim 63.

66. A seed of the transgenic plant of claim 64.

67. A seed of the transgenic plant of claim 65.